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Simple, fast and robust LC-MS/MS method for the simultaneous quantification of canagliflozin, dapagliflozin and empagliflozin in human plasma and urine

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ABSTRACT

Sodium–glucose cotransporter 2 –inhibitors (SGLT2i) are oral glucose-lowering drugs that have also demonstrated cardioprotective and renoprotective effects. SGLT2i play an increasingly important role in the treatment of type 2 diabetes. Here we report a simple and robust liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the simultaneous quantification of three SGLT2i (canagliflozin, dapagliflozin and empagliflozin) in human plasma, serum and urine with a runtime of 1 min. Methanol was used as protein precipitating agent. Chromatographic separation was accomplished using a Waters ACQUITY UPLC HSS T3 1.8 µm; 2.1 × 50 mm column with a Waters ACQUITY UPLC HSS T3 1.8 µm VanGuard Pre-column; 2.1 × 5 mm, using gradient elution with ammonium acetate 20 mM (pH 5) and acetonitrile as mobile phase at a flow rate of 0.8 ml/min. Mass spectrometric analysis of the acetate adduct ions was carried out using electrospray with negative ionization and SRM mode. The assay was validated according to FDA and EMA guidelines, including selectivity, linearity, accuracy and precision, dilution integrity, stability and recovery. With a sample volume of 200 µl, linear ranges of 10–5000 µg/L, 1–500 µg/L and 2–1000 µg/L for canagliflozin, dapagliflozin and empagliflozin respectively, were achieved. The assay was successfully applied in two pharmacokinetic studies with dapagliflozin and empagliflozin. In conclusion, we developed and validated a simple, fast and robust LC-MS/MS method for the simultaneous quantification of canagliflozin, dapagliflozin and empagliflozin, that allows rapid analysis of large numbers of samples and can be used for both pharmacokinetic studies and biomedical analysis of canagliflozin, dapagliflozin and empagliflozin.

1. Introduction

Sodium–glucose cotransporter 2 –inhibitors (SGLT2i) are oral glucose-lowering drugs that are used for the treatment of type 2 diabetes. They selectively and reversibly block the sodium–glucose cotransporter 2 (SGLT2) in the proximal tubule of the kidney which accounts for reabsorption of 90% of filtered glucose [1]. Since SGLT2 mediated glucose reabsorption is coupled to sodium reabsorption, inhibition of the SGLT2 transporter leads to excretion of both glucose and sodium in the urine. Clinically, SGLT2i reduce HbA1c by 7–10 mmol/mol (0.6–0.9%) when compared with placebo [2]. As a result of increased glucosuria and natriuresis, SGLT2i exert diuretic effects which lead to reduction in body weight and blood pressure [3]. Importantly, in large cardiovascular and renal outcome trials, SGLT2i have demonstrated cardioprotective [4–6] and renoprotective effects [7,8]. The adverse effects associated with SGLT2i are generally mild and consistent with the mechanism of action. The most common adverse effects include genital mycotic infections and intravascular volume depletion. Due to its favorable efficacy and safety profile, SGLT2i are playing an increasingly important role in the management of cardiovascular and kidney complications in patients with type 2 diabetes.

Four SGLT2i are currently approved for the treatment of type 2 diabetes by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA): canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin with the former three being most frequently used in clinical practice. Recently, dapagliflozin was also

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approved by the EMA for the treatment of insufficiently controlled type 1 diabetes mellitus as an adjunct to insulin in patients with BMI ≥ 27 kg/m2, when insulin alone does not provide adequate glycemic control despite optimal insulin therapy.

To support further investigation of the pharmacokinetics and pharmacodynamics of SGLT2i, the availability of a bioanalytical assay for the quantification of SGLT2i in human plasma is essential. Because of its selectivity, sensitivity and high throughput, LC-MS/MS is presently the preferred technique for the quantitative determination of drugs in biological matrices. Currently, a limited number of LC-MS/MS methods for the determination of SGLT2i in human plasma has been reported. In some of these methods however, only a single SGLT2i was analyzed [9,10]. Other LC-MS/MS methods have been reported that simultaneously quantify an SGLT2i with other, non-SGLT2i, glucose lowering drugs [11–14]. An assay that simultaneously analyzes multiple SGLT2i leads to more efficiency when studying SGLT2i in clinical practice where patients may receive different SGLT2is. Recently, two LC-MS/MS methods have been reported for the simultaneous quantification of canagliflozin, dapagliflozin and empagliflozin [15,16]. However, these methods have a relatively long runtime of 5 and 9 min respectively, and the applicability of these methods in materials other than plasma is not described.

Here we present the development and validation of a fast, sensitive and robust LC-MS/MS method with 1-minute run times for the simultaneous quantification of canagliflozin, dapagliflozin and empagliflozin in human EDTA plasma, serum and urine suitable for clinical studies.

2. Materials & methods

2.1. Chemicals & reagents

Canagliflozin, dapagliflozin, empagliflozin and the corresponding stable isotope-labeled internal standards (IS) [$^{13}C_6$]- canagliflozin, [$^{13}C_6$]- dapagliflozin and [$^{13}C_6$]- empagliflozin were purchased from Alsachim (Illkirch-Graffenstaden, France). Structural formulas are shown in Fig. 1. MS grade acetonitrile and methanol were obtained from Biosolve (Valkenswaard, The Netherlands). Purified water was generated by a Milli-Q Advantage A10 system (Millipore B.V., Amsterdam, The Netherlands). Ammonium acetate, acetic acid and dimethyl sulfoxide, all of analytical grade, were purchased from Merck (Darmstadt, Germany). Blank human EDTA plasma and blank human urine was supplied by healthy volunteers according to local medical center standard procedures. Drug-free human serum was obtained from Millipore (Temecula, CA, USA).

2.2. Equipment

A Thermo Scientific TSQ Quantiva triple-stage quadrupole mass spectrometer, with a combined Thermo Scientific Vanquish UPLC system was used for all experiments. The Thermo Scientific Vanquish UPLC system was equipped with an autosampler (temperature controlled at 10 °C), a binary LC-pump and a thermostatic column compartment. The Thermo Scientific TSQ Quantiva operated with heated ion electrospray ionization (H-ESI) in the negative ion detection and selected reaction monitoring (SRM) mode.

2.3. Chromatography & mass spectrometric conditions

Chromatographic separation was accomplished using a Waters ACQUITY UPLC HSS T3 1.8 µm; 2.1 × 50 mm column with a Waters ACQUITY UPLC HSS T3 1.8 µm VanGuard Pre-column; 2.1 × 5 mm. The column oven temperature was maintained at 40 °C. Mobile phase A consisted of ammonium acetate 20 mM, adjusted to pH 5 with acetic acid. Mobile phase B consisted of acetonitrile.

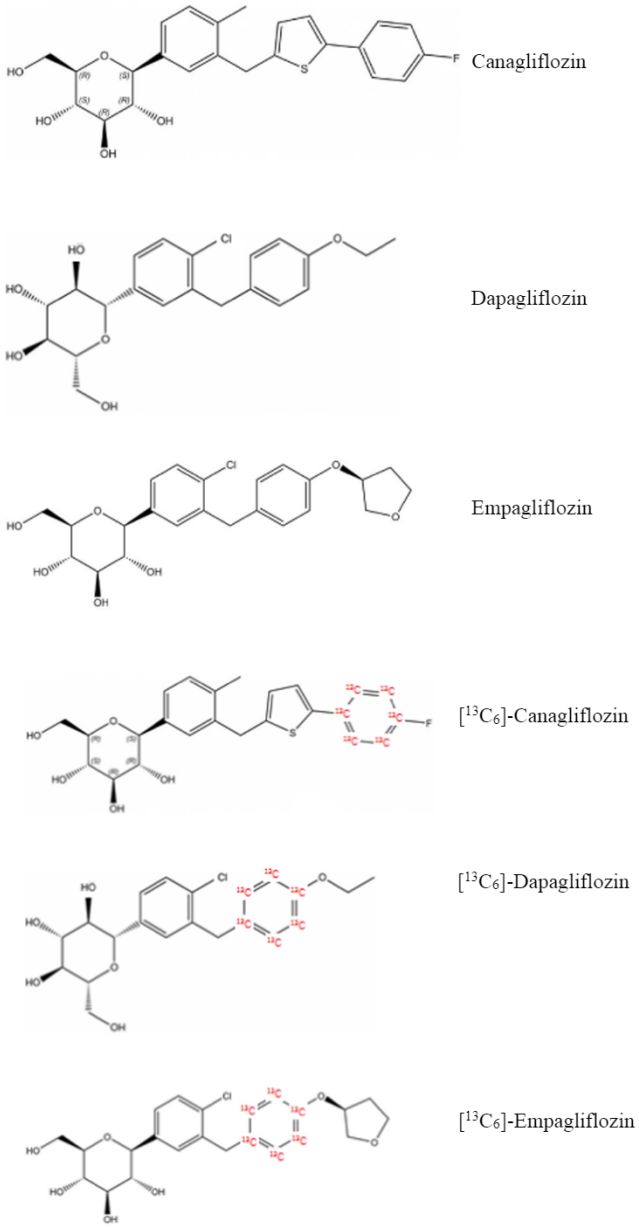

Fig. 1. Structure of canagliflozin, dapagliflozin and empagliflozin and the corresponding stable isotope-labeled internal standards (IS) [$^{13}C_6$]- canagliflozin, [$^{13}C_6$]- dapagliflozin and [$^{13}C_6$]- empagliflozin.

2.4. Preparation of stock solutions, calibration and quality control samples and precipitation reagent

Stock solutions were prepared by dissolving accurately weighed quantities of canagliflozin, dapagliflozin and empagliflozin in dimethyl sulfoxide (DMSO). Separate stock solutions were used for the preparation of calibration samples and the preparation of quality control (QC) samples. Working solutions were prepared by diluting the appropriate amounts of stock solutions with DMSO.

Calibration and QC samples were prepared by spiking the working solutions and stock solutions into blank human EDTA plasma. All samples were stored in the freezer at −20 °C and brought to room temperature before use.

Precipitation reagent was prepared by spiking stock solutions of [$^{13}C_6$]- canagliflozin, [$^{13}C_6$]- dapagliflozin and [$^{13}C_6$]- empagliflozin into methanol to final concentrations of 1000 µg/L, 100 µg/L and 200 µg/L respectively.

2.5. Sample preparation

Of each plasma sample 200 µl was transferred into a 1.5 ml autosampler vial, followed by 500 µl of precipitation reagent. The samples were vortexed for 1 min, kept at −20 °C for 10 min and then vortexed again for 1 min. Subsequently, the samples were centrifuged for 5 min at 9500 RCF. After centrifugation, 10 µl of the supernatant was injected into the LC-MS/MS.

2.6. Method validation

The developed method was validated according to FDA [17] and EMA [18] guidelines, including selectivity, linearity, accuracy and precision, dilution integrity, stability and recovery. Besides these parameters, matrix comparison between human EDTA plasma and human serum and human urine was carried out.

To test method selectivity, six human EDTA plasma samples from different batches were analyzed. The method is proven to be selective if the responses of interfering compounds of the plasma samples were less than 20% of the signal of the analytes at LLOQ and less than 5% of the signal of the internal standards. Assay linearity was evaluated using eight calibration points over a concentration range of 10–5000 µg/L for canagliflozin 1–500 µg/L for dapagliflozin and 2–1000 µg/L for empagliflozin. Linearity was accepted if the back-calculated concentrations of the calibration samples were within 15% of the nominal values (within 20% at LLOQ level). At least six out of the eight calibration points should meet this criterion. Assay accuracy and precision were tested over three separate days by analysis of four QC samples – LLOQ, LOW, MED and HIGH – in fivefold. For each accuracy and precision concentration, bias and CV were calculated per run. Within-run, between run and overall coefficients of variation (CV) were calculated using 1-way analysis of variance (ANOVA). The maximum tolerated bias and CV were 20% for the LLOQ and 15% for all other calibration and QC samples. Dilution integrity was assessed by diluting a QC sample with an over-the-curve concentration ten times with blank human EDTA plasma. As for accuracy and precision, dilution integrity was performed in fivefold on three different days. The stability of canagliflozin, dapagliflozin and empagliflozin was tested under various conditions, including autosampler stability, freeze–thaw stability and stability in the refrigerator (at 5 °C) and at room temperature. The extraction recovery and matrix effects (ME) were determined in six different samples of human EDTA plasma, at QC concentrations LOW and HIGH. To investigate whether the method is also applicable to other human matrices, a matrix comparison was performed by analyzing QC samples in human serum and in human urine in fivefold and calculate the concentrations on the calibration curves in human EDTA plasma. The values for accuracy and precision need to be less than 15% (less than 20% for the LLOQ) to approve the matrix comparison.

2.7. Pharmacokinetic analysis

After validation, the method was used to analyze plasma samples from 2 pharmacokinetic studies with dapagliflozin and empagliflozin. In the first study, patients with non-diabetic kidney disease and macroalbuminuria received dapagliflozin 10 mg once daily. Blood samples were collected after 6 weeks of treatment at t = 0, 30, 60, 90, 120, 150, 180, 210 and 240 min. In the second study, patients with type 2 diabetes and elevated albuminuria were treated with empagliflozin 10 mg once daily. Blood samples were collected after the first dose at t = 0, 15, 30, 45, 60, 90, 120, 240 and 360 min, and after 24 h. Ethical approval for both studies was obtained from the medical ethics committee of each participating center. The studies were conducted in adherence with the Declaration of Helsinki and good clinical practice guidelines. All patients have signed informed consent before any study specific procedure commenced.

Table 1
Elution gradient.

Time (min)	A (%)	B (%)	Flow (mL/min)
0.00	75	25	0.8
0.30	45	55	0.8
0.50	45	55	0.8
0.55	5	95	0.8
0.80	5	95	0.8
0.85	75	25	0.8
1.00	75	25	0.8

3. Results and discussion

3.1. Chromatography & mass spectrometric conditions

During method development, chromatographic conditions have been optimized to achieve good separation of canagliflozin, dapagliflozin and empagliflozin. This resulted in the elution gradient shown in Table 1. With a run time of 1.0 min the retention times for canagliflozin, dapagliflozin and empagliflozin as well as their corresponding stable isotope-labeled internal standards [$^{13}C_6$]- canagliflozin, [$^{13}C_6$]-dapagliflozin and [$^{13}C_6$]- empagliflozin were 0.86 min, 0.74 min and 0.66 min, respectively. This short runtime results in a high throughput method suitable to process many samples and is a clear benefit over previously published methods for the simultaneous quantification of multiple SGLT2i [15,16].

Best sensitivity was accomplished by using negative ionization with the following settings for the mass spectrometric parameters: spray voltage 2900 V; vaporizer temperature 350 °C; ion transfer tube temperature 140 °C; sheath gas pressure 60 arbitrary units and auxiliary gas pressure 17 arbitrary units. For each of the compounds, selected reaction monitoring (SRM) transitions have been optimized to attain good sensitivity and reproducible fragmentation. The most intense parent ions were found to be the acetate-adducts ($[M + CH_3COO]^-$) of canagliflozin, dapagliflozin and empagliflozin and their internal standards, resulting in the SRM transitions summarized in Table 2.

As the parent mass and the SRM transition of [$^{13}C_6$]- canagliflozin are identical to those of empagliflozin, chromatographic separation is crucial for a specific and reliable analytical method. As shown in Fig. 2, the three compounds are well separated with the developed method.

3.2. Method validation

Six different lots of human EDTA plasma were analyzed with and without the addition of the internal standards. The peak heights of interfering components of the six plasma samples have been compared with the peak heights of canagliflozin, dapagliflozin, and empagliflozin at LLOQ and with the peak heights of the internal standards. Co-eluting components from the plasma samples showed peak heights less than 3%, 12% and 8% of the peak heights at LLOQ of canagliflozin, dapagliflozin and empagliflozin, respectively. Furthermore, the signal of possible interferences didn't exceed 0.2% of the signal at the retention times of the internal standards. Based on these results, the assay was

Table 2
SRM transition settings for canagliflozin, dapagliflozin, empagliflozin and the internal standards.

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)
Canagliflozin	503.2	365.0	20
[$^{13}C_6$]-Canagliflozin	509.2	371.0	20
Dapagliflozin	467.2	329.0	19
[$^{13}C_6$]-Dapagliflozin	473.2	335.0	19
Empagliflozin	509.2	371.0	21
[$^{13}C_6$]-Empagliflozin	515.2	377.0	21

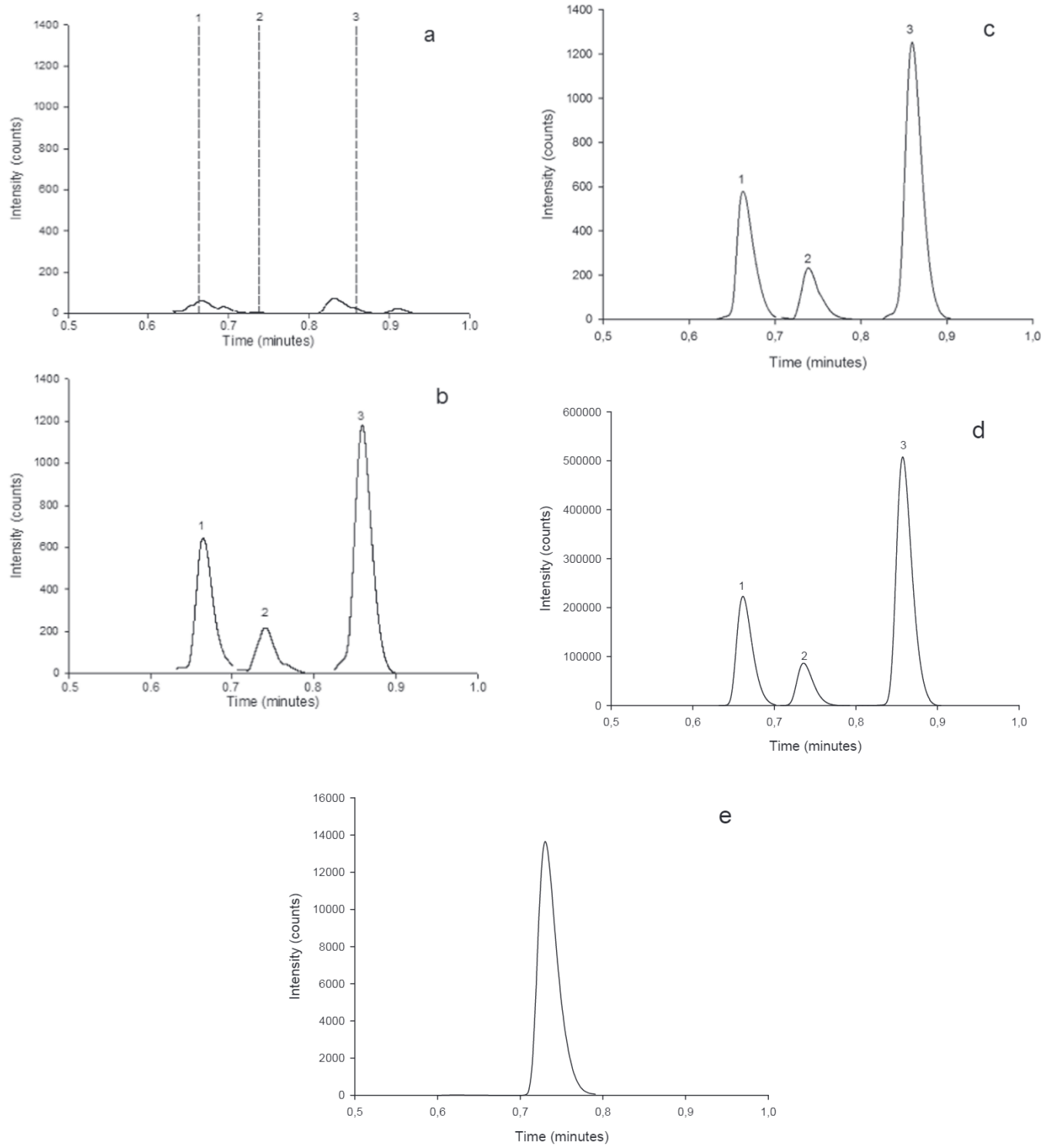

Fig. 2. Selected reaction monitoring (SRM) chromatograms of blank human EDTA plasma (a), the LLOQ in human EDTA plasma (b), the LLOQ in human urine (c) and the HQC in human urine (d) for the analytes empagliflozin (1), dapagliflozin (2) and canagliflozin (3). Panel e shows the chromatogram of dapagliflozin in EDTA plasma, 60 min after administration of a 10 mg dose.

proven to be selective for canagliflozin, dapagliflozin, empagliflozin and their isotope-labeled internal standards.

Calibration curves consisting of eight calibration samples in human EDTA plasma were analyzed on three different days. The concentrations ranges were 10–5000 μg/L (canagliflozin), 1–500 μg/L (dapagliflozin) and 2–1000 μg/L (empagliflozin). Peak height ratios of canagliflozin, dapagliflozin and empagliflozin and the internal standards were calculated. These ratios were fitted versus the concentrations, using linear regression and a weighing factor of $1/x^2$. The biases of the back-calculated concentrations ranged from −6.0% to 8.2% for canagliflozin, from −10.5% to 8.7% for dapagliflozin and from −5.8% to 7.1% for empagliflozin. The mean regression coefficient of the calibration curves were 0.9968, 0.9949 and 0.9979, respectively. As the results met the specified criteria, linearity of the method was proven for all three compounds.

The mean accuracies and the within-run and between-run precision

Table 3
Results of accuracy and precision measurements for QC samples in human EDTA plasma.

Compound	QC Level	Nominal concentration (μg/L)	Accuracy (%)	Precision (CV %)	
				Within-run (n = 5)	Between-run (n = 15)
Canagliflozin	LLOQ	10	97.6	6.6	3.8
	LOW	50	92.9	4.0	1.6
	MED	2000	98.4	0.7	1.1
	HIGH	4000	101.9	0.7	1.1
	DILUTION (factor 10)	2500	99.2	1.0	1.8
Dapagliflozin	LLOQ	1	99.7	13.7	0.0
	LOW	5	94.6	9.1	0.0
	MED	200	97.3	1.5	1.6
	HIGH	400	101.0	1.8	1.5
	DILUTION (factor 10)	250	95.6	1.1	1.4
Empagliflozin	LLOQ	2	101.7	9.9	3.7
	LOW	10	94.6	4.1	3.1
	MED	400	97.7	1.5	1.1
	HIGH	800	101.6	1.0	1.6

of canagliflozin, dapagliflozin and empagliflozin of all QC levels, are presented in Table 3. The accuracies were between 92% and 102% and the within-run and between-run precision was less than 14% and 4% respectively. The accuracy and precision of the QC samples were within the acceptance range of the FDA and EMA criteria.

The ten-fold dilution of an over-the-curve concentrated QC sample, yielded mean accuracies of 99.7% for canagliflozin, 95.6% for dapagliflozin and 97.8% for empagliflozin. The precision (within-run and between-run) was less than 3% for all three compounds. As the criteria were 85%-115% for accuracy and less than 15% for precision, the integrity of a ten-fold dilution was established.

In Table 4, the results of the stability tests of canagliflozin, dapagliflozin and empagliflozin in human EDTA plasma are summarized. The accuracies of the QC samples stored for seven days at room temperature and in the refrigerator ranged from 92% to 102%, with precisions less than 10% for all three analytes. After seven days in the autosampler and after four freeze–thaw cycles, the remaining concentrations were between 95% and 103% of the initial concentrations, with precisions not exceeding 10%. With these results, stability of canagliflozin, dapagliflozin and empagliflozin in human EDTA plasma was proven for seven days at room temperature and in the refrigerator, for seven days in the autosampler after sample preparation and for four freeze–thaw cycles. We did not determine long-term stability at −20 °C and −80 °C. However, research by others has shown stability at −20 °C/−28 °C ranging from 30 to 337 days for canagliflozin [19], dapagliflozin [9] and empagliflozin [20], indicating long-term stability of the analytes in human plasma.

To determine the matrix effect, the matrix factors of six different lots of blank human EDTA plasma spiked with canagliflozin, dapagliflozin and empagliflozin at QC LOW and QC HIGH concentrations, were calculated. The matrix factors for canagliflozin and [$^{13}C_6$]- canagliflozin were 86.3% and 85.8% (QC LOW), and 98.9% and 99.8% (QC HIGH), resulting in IS normalized matrix factors of 100.5% and 99.1% with CVs of 3.3% and 0.9%, respectively. The matrix factors for dapagliflozin and [$^{13}C_6$]- dapagliflozin were 81.4% and 85.3% (QC LOW) and 124.1% and 122.0% (QC HIGH), resulting in IS normalized matrix factors of 95.7% and 101.7%, with CVs of 6.9% and 1.3%, respectively. The matrix factors for empagliflozin and [$^{13}C_6$]- empagliflozin were 88.5% and 89.9% (QC LOW) and 108.6% and 111.1% (QC HIGH), resulting in IS normalized matrix factors of 98.8% and 97.8% with CVs of 3.7% and 1.5%, respectively. As the CVs of all IS normalized matrix factors of the six different lots of blank plasma were less than 15% for all compounds, there was no significant difference between the six batches of blank human EDTA plasma.

The recoveries of canagliflozin and [$^{13}C_6$]- canagliflozin, dapagliflozin and [$^{13}C_6$]- dapagliflozin and empagliflozin and [$^{13}C_6$]- empagliflozin were between 84.8% and 91.1%, from 84.3% to 108.1% and between 88.6% and 101.0%, respectively. All CVs ranged from 2.1% to 13.7%. In conclusion, good and reproducible recoveries of canagliflozin, dapagliflozin and empagliflozin were achieved with the developed sample preparation method.

Finally, matrix comparison of human serum and human urine was performed. The results of these experiments are presented in Table 5. Although the accuracies of the LLOQs of canagliflozin in human serum and of the LLOQ of dapagliflozin in human urine are relatively high, they still meet the requirement for the LLOQ. As the calculated CVs comply with the criteria as well, the assay was found to be suitable for

Table 4
Stability of canagliflozin, dapagliflozin and empagliflozin in human EDTA plasma.

Condition	Period	Compound	QC Level	Nominal concentration (μg/L)	Accuracy (%)	Precision (CV%; n = 5)
Room temperature	7 days	Canagliflozin	LOW	50	92.9	2.1
			HIGH	4000	100.4	1.6
		Dapagliflozin	LOW	5	93.5	9.3
			HIGH	400	100.8	2.3
		Empagliflozin	LOW	10	92.0	3.0
			HIGH	800	100.9	0.9
Refrigerator (5 °C)	7 days	Canagliflozin	LOW	50	92.8	2.9
			HIGH	4000	101.1	0.3
		Dapagliflozin	LOW	5	100.2	9.0
			HIGH	400	100.5	2.3
		Empagliflozin	LOW	10	94.2	5.0
			HIGH	800	102.0	0.4
Autosampler (10 °C)	7 days	Canagliflozin	LOW	50	95.4	1.0
			HIGH	4000	102.0	0.9
		Dapagliflozin	LOW	5	99.0	4.5
			HIGH	400	98.0	1.2
		Empagliflozin	LOW	10	95.6	5.5
			HIGH	800	101.8	0.7
Freeze-thaw (at −20 °C)	4 cycles	Canagliflozin	LOW	50	100.8	4.7
			HIGH	4000	100.5	0.5
		Dapagliflozin	LOW	5	103.0	9.6
			HIGH	400	101.3	1.7
		Empagliflozin	LOW	10	102.0	4.4
			HIGH	800	100.7	0.7

Table 5
Results of matrix comparison measurements for QC samples in human serum and human urine.

Matrix	Compound	QC Level	Nominal concentration (µg/L)	Accuracy (%)	Precision (CV%; n = 5)
Human serum	Canagliflozin	LLOQ	10	119.1	5.2
		LOW	50	97.9	2.5
		MED	2000	97.9	0.9
		HIGH	4000	103.8	1.0
	Dapagliflozin	LLOQ	1	98.8	16.3
		LOW	5	96.8	7.6
		MED	200	94.6	3.4
		HIGH	400	100.8	1.4
	Empagliflozin	LLOQ	2	107.6	5.5
		LOW	10	96.8	5.8
		MED	400	97.1	1.1
		HIGH	800	103.9	0.6
Human urine	Canagliflozin	LLOQ	10	88.7	9.0
		LOW	50	92.1	2.3
		MED	2000	91.7	0.6
		HIGH	4000	93.4	1.0
	Dapagliflozin	LLOQ	1	119.9	8.5
		LOW	5	106.1	11.9
		MED	200	94.2	1.4
		HIGH	400	96.1	1.8
	Empagliflozin	LLOQ	2	104.0	8.7
		LOW	10	104.7	5.7
		MED	400	96.2	1.5
		HIGH	800	99.7	1.7

the quantification of canagliflozin, dapagliflozin and empagliflozin in human serum and human urine too.

3.3. Comparison with reported methods

Recently, two other LC-MS/MS methods for the simultaneous quantification of multiple gliflozins have been reported. The short runtime of the proposed method offers an advantage over these methods, especially when large numbers of samples are to be analyzed. Also, the proposed method was found suitable for the analysis of gliflozins in human serum and urine. A comparison of the analytical performances of the proposed method and the previously reported methods is presented in Table 6.

3.4. Analysis of subject samples

The method was applied to samples from two pharmacokinetic studies with empagliflozin and dapagliflozin. Fig. 3 shows the plasma concentration curves of six patients who received empagliflozin or dapagliflozin, respectively. For both dapagliflozin and empagliflozin, the plasma concentrations found fell well within the validated ranges of the calibration curves.

4. Conclusions

We developed and validated a fast and robust LC-MS/MS method for the simultaneous quantification of canagliflozin, dapagliflozin and empagliflozin in human plasma. The sample pretreatment and assay's simplicity and short run time (1.0 min) allow rapid analysis of large numbers of samples. The assay can be used for both pharmacokinetic studies and biomedical analysis of canagliflozin, dapagliflozin and empagliflozin and is also suitable for the quantification of canagliflozin, dapagliflozin and empagliflozin in human serum and human urine.

Author contributions

A.M.A.W. and D.J.T. developed and validated the method. A.B.vd.A.B. and A.M.A.W. wrote the first draft of the manuscript. H.J.L.H. and D.J.T. revised the manuscript critically for important intellectual content. All authors gave final approval to submit the article for publication.

Declaration of Competing Interest

A.B.vd A.B., D.J.T and A.M.A.W. have nothing to disclose.

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Table 6
Comparative assessment of LC-MS/MS methods for the simultaneous quantification of canagliflozin, dapagliflozin and empagliflozin.

Method	Drug	Linear range (µg/L)	Extraction method	Plasma volume (µL)	Analytical runtime (min)	Matrices
Dias BCL et al. [15]	Canagliflozin	25–5000	PPT with acetic ACN	200	9	Plasma
	Dapagliflozin	10–400				
	Empagliflozin	15–1000				
Shah PA et al. [16]	Canagliflozin	3–3000	Ion-pair SPE with SLS	300	5	Plasma
	Dapagliflozin	0.2–200				
	Empagliflozin	1.5–1500				
Proposed method	Canagliflozin	10–5000	PPT with MET	200	1	Plasma
	Dapagliflozin	1–500				Serum
	Empagliflozin	2–1000				Urine

PPT: protein precipitation, ACN: acetonitrile, SPE: solid phase extraction, SLS: Sodium lauryl sulphate, MET: methanol.

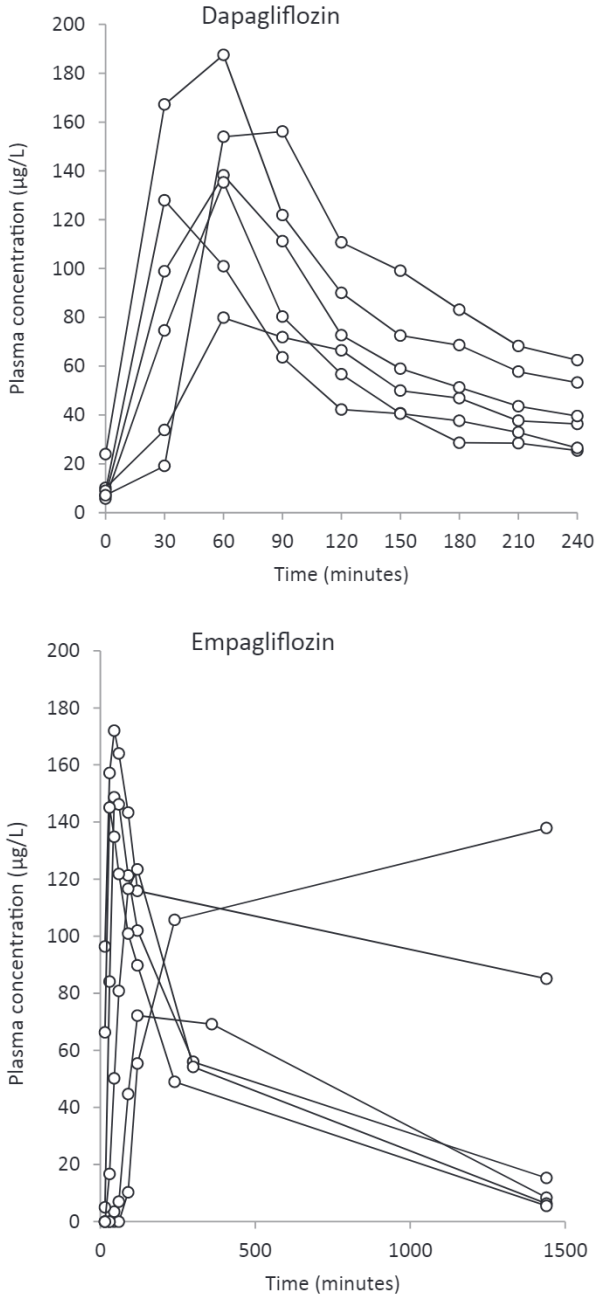

Fig. 3. Plasma concentration – time curves of dapagliflozin and empagliflozin in human EDTA plasma samples.

AstraZeneca, and Janssen (funding directed to his employer).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2020.122257.

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